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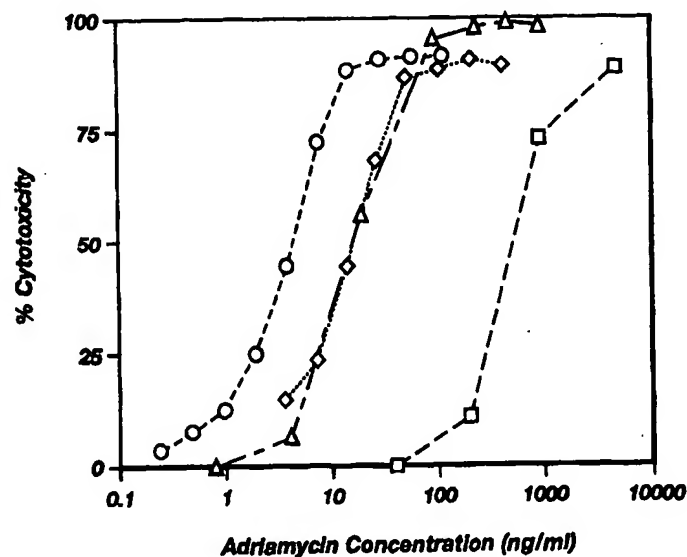
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## (57) Abstract

A composition for intracellular delivery of a chemical agent into an interleukin-2-receptor-bearing cell, e.g. an activated T cell, includes a chemical agent and at least two copies of an interleukin-2-receptor-binding and endocytosis-inducing ligand coupled to a water soluble polymer. The ligand binds to a receptor on the interleukin-2-receptor-bearing cell and elicits endocytosis of the composition. The composition also optionally includes a spacer for coupling the chemical agent and the ligand to the polymer. Chemical agents can include cytotoxins, transforming nucleic acids, gene regulators, labels, antigens, drugs, and the like. A preferred water soluble polymer is polyalkylene oxide, such as polyethylene glycol and polyethylene oxide, and activated derivatives thereof. The composition can further comprise a carrier such as another water soluble polymer, liposome, or particulate. Methods of using these compositions for delivering a chemical agent *in vivo* or *in vitro* are also disclosed.

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**CONJUGATES TARGETED TO THE INTERLEUKIN-2 RECEPTOR**Background of the Invention

5 This invention relates to delivery of chemical agents to cells. More particularly, this invention relates to compositions and methods for intracellular delivery of chemical agents to a specific cell type, i.e. cells bearing the interleukin-2 (IL-2) receptor.

10 Toxins that target cell surface receptors or antigens on tumor cells have attracted considerable attention for treatment of cancer. E.g., I. Pastan & D. FitzGerald, Recombinant Toxins for Cancer Treatment, 254 Science 1173 (1991); Anderson et al., U.S. Patent Nos. 5,169,933 and 5,135,736; Thorpe et al., U.S. Patent No. 5,165,923; Jansen et al., U.S. Patent No. 4,906,469; Frankel, U.S. Patent No. 4,962,188; Uhr et al., U.S. Patent No. 4,792,447; Masuho et al., U.S. Patent Nos. 4,450,154 and 4,350,626. These agents include a cell-targeting moiety, such as a growth factor or an antigen-binding protein, linked to a plant or bacterial toxin. They kill cells by mechanisms different from conventional chemotherapy, thus potentially reducing or eliminating cross resistance to conventional chemotherapeutic agents.

25 Copending U.S. Patent Application Serial No. 08/305,770, filed September 13, 1994, describes compositions and methods for specific intracellular delivery of a chemical agent into a CR2-receptor-bearing cell, e.g. B lymphocytes. The compositions comprise a CR2-receptor-binding and endocytosis-inducing ligand (CBEL) coupled to the chemical agent. The CBEL binds to the CR2 receptor on the surface of B lymphocytes and elicits endocytosis of the composition such that the composition is transported to lysosomes. 35 Optionally, the composition can include a spacer,

which can be either biodegradable (in the lysosome) or non-biodegradable, for coupling the CBEL to the chemical agent. Chemical agents can include cytotoxins, transforming nucleic acids, gene  
5 regulators, labels, antigens, drugs, and the like. The composition can further comprise a carrier such as another water soluble polymer, liposome, or particulate.

Copending U.S. Patent Applications Serial No. 10 08/616,693, filed March 15, 1996, and Serial No. 08/857,009, filed May 15, 1997, describe compositions and methods for specific intracellular delivery of a chemical agent into T lymphocytes. The compositions are represented by the formula  $[L-S]_a-C-[S-A]_b$ , wherein  
15 L is a ligand configured for binding to a receptor on a T lymphocyte and stimulating receptor-mediated endocytosis of the composition, A is a chemical agent, S is a spacer moiety, C is a water soluble polymer having functional groups compatible with forming  
20 covalent bonds with the ligand, chemical agent, and spacer, and a and b are positive integers. These compositions are also designed to be transported to lysosomes. Preferred water soluble polymers include poly(ethylene glycol) and a copolymer of N-(2-  
25 hydroxypropyl)methacrylamide (HPMA). Preferred chemical agents include cytotoxins, transforming nucleic acids, gene regulators, labels, antigens, drugs, and the like. The composition can further comprise a carrier such as other water soluble  
30 polymers, liposomes, or particulates.

It would also be advantageous to develop additional compositions that are specifically targeted to other receptors on T lymphocytes. For example, targeting of T lymphocytes would enable therapeutic  
35 applications for T-cell-associated diseases and tissue

graft rejection. Such T-cell-associated diseases include arthritis, T-cell lymphoma, skin cancers, psoriasis, and diseases resulting from HIV infection.

5 In view of the foregoing, it will be appreciated that compositions for intracellular delivery of chemical agents to T cells and methods of use thereof would be significant advancements in the art.

#### Objects and Summary of the Invention

10 It is an object of the present invention to provide compositions for intracellular delivery of selected chemical agents to a specific cell type, i.e. IL-2-receptor-bearing cells.

15 It is also an object of the invention to provide methods of making and methods of using compositions for intracellular delivery of selected chemical agents to IL-2-receptor-bearing cells.

20 It is another object of the invention to provide compositions and methods for delivering selected chemical agents to IL-2-receptor-bearing cells using water soluble polymers that are inexpensive, FDA-approved, and resistant to development of an antibody response.

25 It is yet another object of the invention to provide compositions and methods of use thereof for intracellular delivery of selected chemical agents to activated T cells.

30 These and other objects are achieved by providing a composition for intracellular delivery of a chemical agent into an IL-2-receptor bearing cell, the composition comprising (a) a water-soluble, biocompatible polymer, (b) the chemical agent covalently, releasably coupled to the polymer, and (c) at least two copies of a ligand comprising an IL-2-

receptor-binding peptide covalently coupled to the polymer.

In a preferred embodiment of the invention, the composition has a formula selected from the group  
5 consisting of  $P-[T_a-L-S_b-A]_c$  and  $[A-S_b]_d-P-[T_a-L]_c$ ,  
wherein L is the ligand; A is the chemical agent; S  
and T are spacers, wherein at least S is  
biodegradable; P is a water soluble polymer having  
functional groups compatible with forming covalent  
10 bonds with the ligand; a and b are integers of 0 or 1;  
c is an integer of at least 2; and d is an integer of  
at least 1.

Preferably, P is a polyalkylene oxide. Preferred  
polyalkylene oxides are selected from the group  
15 consisting of alpha-substituted polyalkylene oxide  
derivatives, polyethylene glycol (PEG) homopolymers,  
polypropylene glycol homopolymers, alkyl-capped  
polyethylene oxides, bis-polyethylene oxides,  
copolymers of poly(alkylene oxides), and block  
20 copolymers of poly(alkylene oxides) or activated  
derivatives thereof. Preferably, the polyalkylene  
oxide has a molecular weight of about 200 to about  
50,000. More preferably, the polyalkylene oxide has a  
molecular weight of about 2,000 to about 20,000. Most  
25 preferably, the polyalkylene oxide has a molecular  
weight of about 5,000. Especially preferred  
polyalkylene oxides are polyethylene glycol and  
polyethylene oxide.

The IL-2-receptor-binding peptide is preferably a  
30 member selected from the group consisting of SEQ ID  
NO:1 and biologically functional equivalents thereof.  
More preferably, the IL-2-receptor-binding peptide is  
a member selected from the group consisting of SEQ ID  
NO:1 through SEQ ID NO:11.

The chemical agent is preferably selected from the group consisting of cytotoxins, transforming nucleic acids, gene regulators, labels, antigens, and drugs.

5            Preferably, the spacer comprises a peptide. A preferred peptide spacer comprises Gly-Phe-Leu-Gly (SEQ ID NO:21).

          In one preferred embodiment, the composition further comprises a carrier selected from the group  
10        consisting of other water soluble polymers, liposomes, and particulates. Preferably, such water soluble polymers are selected from the group consisting of dextran, inulin, poly(L-lysine) with modified epsilon amino groups, poly(L-glutamic acid), and N-substituted  
15        methacrylamide-containing polymers.

          A method of delivering a chemical agent *in vitro* into an IL-2-receptor-bearing cell in a heterogeneous population of cells, comprises the steps of:

          (a) providing a composition comprising (i) a  
20        water-soluble, biocompatible polymer, (ii) the chemical agent covalently, releasably coupled to the polymer, and (iii) at least two copies of a ligand comprising an IL-2-receptor-binding peptide covalently coupled to the polymer; and

25        (b) contacting the population of cells with an effective amount of the composition under conditions wherein the ligand binds to an IL-2 receptor on the IL-2-receptor-bearing cell and elicits endocytosis of the composition.

30        A method of delivering a chemical agent intracellularly into an IL-2-receptor-bearing cell in a warm-blooded animal, comprising the steps of:

          (a) providing a composition comprising (i) a  
35        water-soluble, biocompatible polymer, (ii) the chemical agent covalently, releasably coupled to the

polymer, and (iii) at least two copies of a ligand comprising an IL-2-receptor-binding peptide covalently coupled to the polymer; and

(b) systemically administering to the warm-blooded animal an effective amount of the composition under conditions wherein the ligand contacts and binds to an IL-2 receptor on the IL-2-receptor-bearing cell and elicits endocytosis of the composition.

10

#### Brief Description of the Drawings

FIG. 1 shows the *in vitro* cytotoxic activity of a composition according to the present invention and control compositions against human HSB-2 T cells: (□) PEG-GFLG-ADR (SEQ ID NO:21); (Δ) PEG-TT13-ADR (SEQ ID NO:13); (◇) PEG-TT7-ADR (SEQ ID NO:14); and unconjugated adriamycin.

15

#### Detailed Description of the Invention

Before the present compositions and methods for targeted delivery to IL-2-receptor-bearing cells are disclosed and described, it is to be understood that this invention is not limited to the particular embodiments, process steps, and materials disclosed herein as such embodiments, process steps, and materials may vary somewhat. It is also to be understood that the terminology used herein is used for the purpose of describing particular embodiments only and is not intended to be limiting since the scope of the present invention will be limited only by the appended claims and equivalents thereof.

25

30

It must be noted that, as used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus,

35



for example, reference to a composition containing "a ligand" includes reference to two or more ligands, reference to "a chemical agent" includes reference to one or more of such chemical agents that may be the same or different chemical agents, and reference to "a spacer" includes reference to two or more spacers.

In describing and claiming the present invention, the following terminology will be used in accordance with the definitions set out below.

As used herein, "peptide" means peptides of any length and includes proteins. The terms "polypeptide" and "oligopeptide" are used herein without any particular intended size limitation, unless a particular size is otherwise stated.

As used herein, "IL-2-receptor-binding peptide" means a peptide configured for binding to an IL-2 receptor and stimulating internalization thereof by receptor-mediated endocytosis. According to the present invention, ligands comprising such IL-2-receptor-binding peptides are coupled to various functional molecules so that upon endocytosis of the ligands the various functional molecules coupled thereto are also internalized by the cells.

Preferred IL-2-receptor-binding peptides include the peptide having the amino acid sequence identified as SEQ ID NO:1 and biologically functional equivalents thereof. Such functional equivalents retain functionality in binding the IL-2 receptor and eliciting receptor-mediated endocytosis although they may be truncations, deletion variants, or substitution variants of SEQ ID NO:1 or include additional amino acid residues attached thereto. It is also preferred that the IL-2-receptor-binding peptides have a size of about 6-20 amino acid residues, more preferably about

6-12 amino acid residues, and most preferably about 6-8 amino acid residues.

As mentioned above, changes may be made in the structure of the IL-2 receptor-binding peptide while  
5 maintaining the desirable receptor-binding characteristics. For example, certain amino acid residues may be substituted for other amino acid residues in a protein structure without appreciable loss of interactive binding capacity with structures  
10 such as, for example, antigen-binding regions of antibodies or binding sites of ligands such as an IL-2 receptor-binding peptide. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain  
15 amino acid sequence substitutions can be made in a protein sequence and nevertheless obtain a protein with like properties. It is thus contemplated that various changes may be made in the sequence of an IL-2 receptor-binding peptide without appreciable loss of  
20 its biological utility or activity.

It is also well understood by the skilled artisan that inherent in the definition of a biologically functional equivalent protein or peptide is the concept that there is a limit to the number of changes  
25 that may be made within a defined portion of the molecule and still result in a molecule with an acceptable level of equivalent biological activity. It is also well understood that where certain residues are shown to be particularly important to the  
30 biological or structural properties of a protein or peptide, e.g. residues in active sites, such residues may not generally be exchanged.

Amino acid substitutions are generally based on the relative similarity of the amino acid side-chains  
35 relative to, for example, their hydrophobicity,

hydrophilicity, charge, size, and the like. An analysis of the size, shape, and type of the amino acid side-chains reveals, for example, that arginine, lysine, and histidine are all positively charged residues; that alanine, glycine, and serine are all a similar size; and that phenylalanine, tryptophan, and tyrosine all have a generally similar shape. Therefore, based upon these considerations, the following conservative substitution groups or biologically functional equivalents have been defined: (a) Cys; (b) Phe, Trp, Tyr; (c) Gln, Glu, Asn, Asp; (d) His, Lys, Arg; (e) Ala, Gly, Pro, Ser, Thr; and (f) Met, Ile, Leu, Val. M. Dayhoff et al., Atlas of Protein Sequence and Structure (Nat'l Biomed. Res. Found., Washington, D.C., 1978), hereby incorporated by reference.

To effect more quantitative changes, the hydropathic index of amino acids may be considered. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics, which are as follows: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is generally understood in the art. J. Kyte & R. Doolittle, A simple method for displaying the hydropathic character of a protein, 157 J. Mol. Biol. 105-132 (1982), incorporated herein by reference. It is known that certain amino acids may be substituted

for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making changes based on the hydropathic index, the substitution of amino acids whose  
5 hydropathic indices are within  $\pm 2$  is preferred, within  $\pm 1$  is particularly preferred, and within  $\pm 0.5$  is even more particularly preferred.

It is also understood that an amino acid can be substituted for another having a similar  
10 hydrophilicity value and still obtain a biologically equivalent protein. As detailed in U.S. Patent No. 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0); glutamate (+3.0  $\pm 1$ );  
15 serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5  $\pm 1$ ); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine  
20 (-2.5); tryptophan (-3.4).

In making changes based upon similar hydrophilicity values, the substitution of amino acids whose hydrophilicity values are within  $\pm 2$  is preferred, within  $\pm 1$  is particularly preferred, and  
25 within  $\pm 0.5$  is even more particularly preferred.

A hexapeptide believed to be a part of IL-2 that binds to the IL-2 receptor has been identified (SEQ ID NO:1), D.A. Weigent et al., 139 Biochem. Biophys. Res. Commun. 367-74 (1986). Moreover, regions of homology  
30 between this IL-2 hexapeptide and env proteins of immunosuppressive retroviruses have been discovered. D.A. Weigent et al., *supra*; W.E. Reiher III et al., 83 Proc. Nat'l Acad. Sci. USA 9188-92 (1986). Thus, amino acid substitutions in these regions of homology  
35 as compared to the IL-2 hexapeptide are also

considered to be biologically functional equivalents. Therefore, illustrative biologically functional equivalents of SEQ ID NO:1 include the following: SEQ ID NO:2; SEQ ID NO:3; SEQ ID NO:4; SEQ ID NO:5; and  
5 SEQ ID NO:6. Other illustrative biologically functional equivalents have also been discovered, including: SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:9; SEQ ID NO:10; and SEQ ID NO:11. Additional biologically functional equivalents can be discovered by a person  
10 of ordinary skill in the art according to the guidance and principles disclosed herein without undue experimentation.

As used herein, "macromolecule" means a composition comprising a water soluble polymer with a  
15 ligand and a chemical agent bound thereto. Preferably the polymer is a polyalkylene oxide and the ligand is an oligopeptide. The chemical agent can be from many different classes of molecules, as explained in more detail herein.

20 As used herein, "prodrug" means a chemical agent that is chemically modified to overcome a biological barrier. When a chemical agent is converted into its prodrug form, its biological activity is eliminated or substantially reduced, but the biological barrier that  
25 inhibited its effectiveness is no longer problematic. The chemical group that is attached to the chemical agent to form the prodrug, i.e. the "pro-moiety", is removed from the prodrug by enzymatic or nonenzymatic means to release the active form of the chemical  
30 agent. See A. Albert, Chemical Aspects of Selective Toxicity, 182 Nature 421 (1958). The instant compositions are prodrugs because the chemical agent that has the selected effect when internalized in IL-2-receptor-bearing cells is modified with a ligand,  
35 water soluble polymer, and, optionally, spacers such

that the composition is delivered into the IL-2-receptor-bearing cells, thus penetrating the cell membrane thereof. The biological effect of the chemical agent is greatly reduced or eliminated until  
5 the composition is delivered intracellularly and the chemical agent is released from the remainder of the composition by biodegradation of the spacer.

As used herein, "chemical agent" means and includes any substance that has a selected effect when  
10 internalized into an IL-2-receptor-bearing cell. Certain chemical agents have a physiological effect, such as a cytotoxic effect or an effect on gene regulation, when internalized into the cell. A "transforming nucleic acid" (RNA or DNA), when  
15 internalized into a cell, can be replicated and/or expressed within the cell. Other nucleic acids can interact with regulatory sequences or regulatory factors within the cell to influence gene expression within the cell in a selected manner. A detectable  
20 label delivered intracellularly can permit identification of cells that have internalized the compositions of the present invention by detection of the label. Drugs or pharmacologically active compounds can be used to ameliorate pathogenic effects  
25 or other types of disorders. Particularly useful chemical agents include polypeptides, and some such chemical agents are active fragments of biologically active proteins, or are specific antigenic fragments (e.g., epitopes) of antigenic proteins. Thus, chemical  
30 agents include cytotoxins, gene regulators, transforming nucleic acids, labels, antigens, drugs, and the like.

As used herein, "drug" or "pharmacologically active agent" means any chemical material or compound  
35 suitable for intracellular administration in a IL-2

receptor bearing cell, e.g. an activated T lymphocyte, that stimulates a desired biological or pharmacological effect in such cell.

As used herein, "carrier" means water soluble polymers, particulates, or liposomes to which a composition according to the instant invention can be coupled. Such carriers increase the molecular size of the compositions and may provide added selectivity and/or stability. Such selectivity arises because carrier-containing compositions are too large to enter cells by passive diffusion, and thus are limited to entering cells through receptor-mediated endocytosis. The potential for use of such carriers for targeted drug delivery has been established. See, e.g., J. Kopecek, 5 Biomaterials 19 (1984); E. Schacht et al., Polysaccharides as Drug Carriers, in Controlled-Release Technology 188 (P.I. Lee & W.R. Good, eds., 1987); F. Hudecz et al., Carrier design: Cytotoxicity and Immunogenicity of Synthetic Branched Polypeptides with Poly(L-lysine) Backbone, 19 J. Controlled Release 231 (1992); Z. Brich et al., Preparation and Characterization of a Water Soluble Dextran Immunoconjugate of Doxorubicin and the Monoclonal Antibody (ABL364), 19 J. Controlled Release 245 (1992). Thus, illustrative water soluble polymers include dextran, inulin, poly(L-lysine) with modified epsilon-amino groups, poly(L-glutamic acid), N-substituted methacrylamide-containing synthetic polymers and copolymers, and the like.

As used herein, "effective amount" is an amount sufficient to produce a selected effect. For example, a selected effect of a composition containing a cytotoxin as the chemical agent could be to kill a selected proportion of IL-2-receptor-bearing cells, e.g. activated T cells, within a selected time period.

An effective amount of the composition would be the amount that achieves this selected result, and such an amount could be determined as a matter of routine by a person skilled in the art.

5           The compositions of the present invention provide intracellular delivery of a chemical agent capable of eliciting a selected effect when delivered intracellularly into an IL-2-receptor-bearing cell. Illustrative embodiments of the composition have a  
10           formula selected from the group consisting of  $P-[T_a-L-S_b-A]_c$  and  $[A-S_b]_d-P-[T_a-L]_c$ , wherein L is a ligand configured for binding to an IL-2 receptor on the IL-2-receptor-bearing cell and stimulating receptor-mediated endocytosis of the composition; A is  
15           the chemical agent; S and T are spacers, wherein at least S is biodegradable; P is a water soluble polymer having functional groups compatible with forming covalent bonds with the ligand; a and b are integers of 0 or 1; c is an integer of at least 2; and d is an  
20           integer of at least 1. Preferably, c is an integer of 2 to about 1000. The spacers are preferably biodegradable such that the chemical agent is detached from the composition by hydrolysis and/or enzymatic cleavage inside IL-2-receptor-bearing cells, e.g. T  
25           cells, especially in lysosomes. Once detached, the chemical agent can exert its functional effect in the cell. Illustrative of such spacers is the peptide Gly-Phe-Leu-Gly (SEQ ID NO:21). Equivalent peptide spacers are well known in the art. The chemical agent  
30           is selected from the group consisting of cytotoxins, transforming nucleic acids, gene regulators, labels, antigens, drugs, and the like. The water soluble polymer (represented by P in the formula above) is preferably a poly(alkylene oxide). Within this group  
35           of substances are alpha-substituted polyalkylene oxide



derivatives, such as methoxypolyethylene glycols or other suitable alkyl-substituted derivatives, such as those containing C<sub>1</sub>-C<sub>4</sub> alkyl groups. It is preferred that the polymer be a monomethyl-substituted PEG homopolymer. Other poly(alkylene oxides) are also useful, including other polyethylene glycol (PEG) homopolymers, polypropylene glycol homopolymers, other alkyl-capped polyethylene oxides, bis-polyethylene oxides, copolymers of poly(alkylene oxides), and block copolymers of poly(alkylene oxides) or activated derivatives thereof. In those aspects of the invention where PEG-based polymers are used, it is preferred that they have molecular weights of from about 200 to about 50,000. Molecular weights of about 2,000 to about 20,000 are preferred, and molecular weights of about 5,000 are particularly preferred. PEG is preferred because it is inexpensive, approved by the FDA for administration to humans, and is resistant to eliciting an antibody response.

Poly(ethylene oxide) (PEO) is another preferred water soluble polymer represented by P. The coupling of a ligand to a chemical agent can be, without limitation, by covalent bond, electrostatic interaction, hydrophobic interaction, physical encapsulation, and the like. The compositions of the present invention can further comprise a carrier selected from the group consisting of other water soluble polymers, liposomes, and particulates. Such water soluble polymers for use as carriers are selected from the group consisting of dextran, inulin, poly(L-lysine) (PLL) with modified epsilon amino groups, poly(L-glutamic acid) (PGA), N-substituted methacrylamide-containing polymers and copolymers, and the like. A preferred water soluble polymer is a copolymer of N-(2-hydroxypropyl)methacrylamide (HPMA).

Thus, according to the invention, the composition provides means for preferential binding to an IL-2 receptor, such as on activated T cells, thus triggering internalization of the composition by endocytosis. The chemical agent provides means for achieving a selected effect in the IL-2-receptor bearing cells. Accordingly, for example, chemical agents comprise cytotoxins, including radionuclides, for selective killing or disabling of cells; nucleic acids for genetically transforming or regulating gene expression in cells; drugs or other pharmacologically active agents for achieving a selected therapeutic effect; labels, including fluorescent, radioactive, and magnetic labels, for permitting detection of cells that have taken up the compositions; and the like.

IL-2 is a lymphocyte growth factor produced by T cells that is essential for a normal immune response. Binding of IL-2 to the IL-2 receptor precedes internalization by receptor-mediated endocytosis. The human IL-2 gene has been sequenced, T. Taniguchi et al., 302 Nature 305-10 (1983), hereby incorporated by reference, as has the gene for the human IL-2 receptor, W.J. Leonard et al., 311 Nature 626-31 (1984); T. Nikaido et al., 311 Nature 631-35 (1984); D. Cosman et al., 312 Nature 768-71 (1984). The IL-2 receptor is a heterotrimeric glycoprotein complex on the cell membrane with a 55 kDa  $\alpha$  subunit, a 75 kDa  $\beta$  subunit, and a 64 kDa  $\gamma$  subunit. The only normal human tissues expressing the  $\alpha$  and  $\beta$  subunits are activated T cells, B cells, LGL cells, and monocytes and some liver Kupffer cells, macrophages, and skin Langerhans' cells. A.E. Frankel et al., 11 Leukemia 22-30 (1997). A variety of hematologic neoplasms may show high affinity IL-2 receptor expression including hairy cell leukemia, adult T cell leukemia, and a

fraction of cutaneous T cell lymphomas and B cell chronic lymphocytic leukemias. Recombinant toxins targeted to the IL-2 receptor have been described wherein the ligand is IL-2. A.E. Frankel et al.,  
5 *supra*; U.S. Patent No. 4,675,382; J. vanderSpek et al., 268 J. Biol. Chem. 12077-82 (1993); I. Pastan & D. FitzGerald, *supra*.

In some embodiments of the present invention, the compositions are constructed by chemically conjugating  
10 the ligand and chemical agent to the water soluble polymer. "Chemically conjugating" the ligand and the chemical agent to the water soluble polymer, as that term is used herein, means covalently bonding the ligand and chemical agent to each other, preferably by  
15 way of a spacer moiety, and conjugating the resulting ligand/agent conjugate to the water soluble polymer. In particular embodiments, a spacer moiety is used to form a linkage between the ligand and the chemical agent.

20 Peptide portions of the compositions of the present invention can be produced in a genetically engineered organism, such as *E. coli*, as a "fusion protein." That is, a hybrid gene containing a sequence of nucleotides encoding a ligand, spacer, or  
25 peptide chemical agent can be constructed by recombinant DNA technology. This hybrid gene can be inserted into an organism such that the "fusion protein" encoded by the hybrid gene is expressed. The fusion protein can then be purified by standard  
30 methods, including affinity chromatography. Peptides containing a ligand, spacer, or peptide chemical agent can also be constructed by chemical synthesis. Short peptide ligands are generally preferred, both because short peptides can be manipulated more readily and  
35 because the presence of additional amino acids

residues, and particularly of substantial numbers of additional amino acids residues, may interfere with the function of the peptide ligand in stimulating internalization of the chemical agent by endocytosis.

5           Compositions according to the present invention preferably also further include a protease digestion site, preferably in the spacer moiety, situated such that once the composition is within the cell, such as in a lysosome, the chemical agent can be separated  
10           from the remainder of the composition by proteolysis of the digestion site. Such a protease susceptible spacer can be added regardless of whether the peptide portions of the composition are synthesized chemically or as expression peptides in a genetically engineered  
15           organism. In the latter case, nucleotides encoding the protease susceptible spacer can be inserted into the hybrid gene encoding the ligand and or a peptide chemical agent by techniques well known in the art. In one illustrative embodiment, the protease-  
20           susceptible spacer is designed to be cleaved by proteolysis in the lysosome of the target cell. The composition that is internalized by endocytosis is packaged in an endocytic vesicle, which is transported to a lysosome. Once in the lysosome, the protease-  
25           susceptible spacer is cleaved, and the chemical agent is then available to be transported to the cytoplasm.

          Another aspect of the present invention features a method for specifically effecting a desired activity in IL-2-receptor-bearing cells, e.g. activated T  
30           lymphocytes, contained in a heterogeneous population of cells, by the step of contacting the population of cells with a composition, prepared according to the present invention, that directs such activity intracellularly. The compositions of the invention  
35           are selectively bound to IL-2-receptor-bearing T cells

in the mixed population, whereupon endocytosis of the composition into such activated T cells is stimulated, and the chemical agent effects its activity within such T cells.

5           This application employs, except where otherwise indicated, standard techniques for manipulation of peptides and for manipulation of nucleic acids for expression of peptides. Techniques for conjugation of oligopeptides and oligonucleotides are known in the art, and are described for example in T. Zhu et al., 3  
10           Antisense Res. Dev. 265 (1993); T. Zhu et al., 89 Proc. Nat'l Acad. Sci. USA 7934 (1992); P. Rigaudy et al., 49 Cancer Res. 1836 (1989), which are hereby incorporated by reference.

15           As is noted above, the invention features peptides, employed as ligands, spacers, and/or chemical agents. The peptides according to the invention can be made by any of a variety of techniques, including organic synthesis and  
20           recombinant DNA methods. Techniques for chemical synthesis of peptides are described, for example, in B. Merrifield et al., 21 Biochemistry 5020 (1982); Houghten, 82 Proc. Nat'l Acad. Sci. USA 5131 (1985); M. Bodanszky & A. Bodanszky, The Practice of Peptide  
25           Synthesis (Springer-Verlag 2d ed., 1994), incorporated herein by reference. Techniques for chemical conjugation of peptides with other molecules are known in the art.

          A fusion protein according to the invention can  
30           be made by expression in a suitable host cell of a nucleic acid containing an oligonucleotide encoding a ligand and/or spacer and/or chemical agent. Such techniques for producing recombinant fusion proteins are well-known in the art, and are described generally  
35           in, e.g., J. Sambrook et al., Molecular Cloning: A

Laboratory Manual (2d ed., 1989), the pertinent parts of which are hereby incorporated herein by reference. Reagents useful in applying such techniques, such as restriction endonucleases and the like, are widely known in the art and commercially available from any of several vendors.

Construction of compositions according to the present invention will now be described, with particular reference to examples in which a peptide ligand coupled to a biodegradable spacer (SEQ ID NO:21) and a cytotoxic chemical agent, adriamycin, are coupled to PEG.

#### Example 1

NH<sub>2</sub>-Gly-Leu-OH (Sigma Chemical Co. St. Louis, MO; 2.74 g, 14.6 mmol) was dissolved in 30 ml of phosphate-buffered saline (PBS) containing 0.5 M NaCl. The reaction mixture was stirred and 5 g of solid activated PEG (succinimidyl ester of 5 pendent polyethylene glycol propionic acid; p-5-SPA-5000; Shearwater Polymers, Inc., Huntsville, AL) was added to the solution. The reaction was continued for 4.5 hours at pH 7-8. The reaction mixture was then extracted four times with 500 ml each of dichloromethane (A.C.S., HPLC grade, Sigma or Aldrich, Milwaukee, WI). An emulsion was formed during extraction and was partially broken by adding NaCl to the emulsion. The organic layers were pooled and dried over MgSO<sub>4</sub> overnight. The solution was then filtered, and the filtrate was concentrated by evaporating the dichloromethane with a rotary evaporator at about 35°C using a water pump. The final volume of solution was reduced to about 15 ml. The solution was added to ether (750 ml; anhydrous, Fisher Scientific), and the product (PEG-Gly-Leu-OH)

was precipitated. The precipitates were filtered, washed with ether, and dried in air.

PEG-Gly-Leu-OH (3.5 g, 3.4 mmol) and p-nitrophenol were dissolved in 50 ml of tetrahydrofuran (anhydrous, Aldrich) and 10 ml of ethylacetate (Aldrich). The solution was cooled in an ice bath, and dicyclohexylcarbodiimide (DCC, Sigma; 2.4 g, 11.5 mmol) was added to the reaction mixture in four aliquots. The reaction solution was stirred for 30 minutes in the ice bath. The temperature of the reaction solution was then raised to room temperature, and the reaction was then continued for another 91 hours. The reaction solution was then filtered through filter paper, and the filtrate was concentrated by evaporating the solvent with a rotary evaporator using a water pump. The clear concentrated solution (30 ml) was added to ether (750 ml). The precipitate was filtered, washed in ether, and dried in air. An aliquot of the product was dissolved in 0.1 N NaOH, and the concentration of the liberated p-nitrophenol was estimated by spectrophotometry at 400 nm using a molar extinction coefficient of  $\epsilon = 1.8 \times 10^4$  l/mol-cm. The product (PEG-Gly-Leu-ONp) was determined to have an ONp content of 375  $\mu\text{mol/g}$ .

The product (PEG-Gly-Leu-ONp; 430.88 mg, ONp content 161.2  $\mu\text{mol}$ ) was dissolved in 5 ml anhydrous dimethylformamide (DMF), and a peptide (Gln-His-Leu-Phe-Leu-Gly, SEQ ID NO:12) was added to the solution. About 150  $\mu\text{l}$  of triethylamine diluted 1:2 with DMF was added to the reaction mixture four times in an interval of 15 minutes, and the solution was stirred for 17 hours at room temperature. The reaction solution was added to cold ether (400 ml), and the conjugate precipitates (PEG-TT13-OH; SEQ ID NO:13) were filtered, washed with 400 ml ether, and dried.

Adriamycin (63.97 mg, 111.5  $\mu$ mol, Sigma) and PEG-TT13-OH (450.26 mg, 111.5  $\mu$ mol) were dissolved in 5 ml DMF and DCC solid (59.3 mg) was added to the solution. The reaction was carried out for 22 hours and then  
5 filtered through Whatman No. 4 paper. The precipitate on the filter paper was dried under vacuum and then dissolved in PBS buffer and dialyzed against PBS overnight. After a change of buffer, the solution was dialyzed for 2 additional hours. Adriamycin content  
10 was determined by spectrophotometry at 490 nm. The resulting conjugate had the structure PEG-Gly-Leu-Gln-His-Leu-Phe-Leu-Gly-Adriamycin (hereinafter, "PEG-TT13-ADR;" SEQ ID NO:13).

#### 15 Example 2

In this example, a control composition having the structure PEG-Gly-Phe-Leu-Gly-ADR (hereinafter, "PEG-GFLG-ADR;" SEQ ID NO:21) was prepared according to the procedure of Example 1.

20

#### Example 3

In this example, a composition having the structure PEG-Gly-Leu-Glu-Arg-Ile-Leu-Leu-Gly-Phe-Leu-Gly-Adriamycin (hereinafter, "PEG-TT7-ADR;" SEQ ID  
25 NO:14) was prepared according to the procedure of Example 1.

#### Example 4

In this example, a composition having the  
30 structure PEG-Gly-Leu-Glu<sup>tBt</sup>-His-Ile-Leu-Leu-Gly-Phe-Leu-Gly-Adriamycin (SEQ ID NO:15), where tBt is a tert-butyl group coupled to the COOH side-chain of the glutamic acid residue, was prepared according to the procedure of Example 1. The tert-butyl derivative of  
35 glutamic acid was purchased commercially (Bachem, King



of Prussia, PA) and was incorporated into the oligopeptide during peptide synthesis. The tert-butyl group blocks the COOH group to prevent reaction of the COOH side-chain of the glutamic acid residue with an NH<sub>2</sub> group of adriamycin.

#### Example 5

In this example, a composition having the structure PEG-Gly-Leu-Gln-His-Ile-Leu-Leu-Gly-Phe-Leu-Gly-Adriamycin (SEQ ID NO:16) was prepared according to the procedure of Example 1.

#### Example 6

In this example, a composition having the structure PEG-Gly-Leu-Asp-His-Ile-Phe-Leu-Gly-Phe-Leu-Gly-Adriamycin (SEQ ID NO:17) is prepared according to the procedure of Example 1.

#### Example 7

In this example, a composition having the structure PEG-Gly-Leu-Asn-His-Ile-Phe-Leu-Gly-Phe-Leu-Gly-Adriamycin (SEQ ID NO:18) is prepared according to the procedure of Example 1.

#### Example 8

In this example, a composition having the structure PEG-Thr-Gly-Leu-Gln-His-Ile-Leu-Leu-Gly-Phe-Leu-Gly-Adriamycin (SEQ ID NO:19) is prepared according to the procedure of Example 1.

#### Example 9

In this example, a composition having the structure PEG-Ser-Leu-Gln-His-Ile-Leu-Leu-Gly-Phe-Leu-Gly-Adriamycin (SEQ ID NO:20) is prepared according to the procedure of Example 1.

## Example 10

The *in vitro* effects of PEG-TT13-ADR prepared according to the procedure of Example 1, PEG-GFLG-ADR prepared according to the procedure of Example 2, PEG-TT7-ADR prepared according to the procedure of Example 3, and unconjugated adriamycin were tested on human HSB-2 T cells (ATCC No. CCL 120.1) as follows. Triplicate samples of  $1 \times 10^5$  cells each were mixed with different concentrations of the purified compositions in 0.1 ml of culture medium (RPMI 1640, 10% fetal calf serum) in the wells of a 96-well microtiter plate (Falcon Microtest 111), and incubated for 48 hours at 37°C in a humidified, 5% CO<sub>2</sub> atmosphere. Thereafter, cell viability was assessed by a colorimetric method using the tetrazolium compound MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) and an electron coupling reagent, PMS (phenazine methosulfate). A.J. Cory et al., 3 Cancer Commun. 207 (1991); T.L. Riss & R.A. Moravec, 3 Mol. Biol. Cell. 184a (Supp.; 1992); T.M. Buttke et al., 157 J. Immunol. Methods 233 (1993), hereby incorporated by reference. MTS is bio-reduced by living cells into a soluble formazan product. The absorbance of the formazan at 490 nm can be measured directly from 96 well assay plates without additional processing. The quantity of formazan product as measured by the absorbance at 490 nm is directly proportional to the number of living cells in culture. Reagents for the MTS assay were obtained from Promega Corp. (Madison, Wisconsin). According to this method, 20 µl of MTS/PMS solution (Promega No. G-5421) was added to each well of the assay plate. The plate was then further incubated at 37°C in a humidified, 5% CO<sub>2</sub> atmosphere for 4 hours. The absorbance of each well

was then measured at 490 nm with an EL311 Microplate Autoreader (Bio-Tek Instruments). The mean absorbance for each treatment was then calculated, and the percent cytotoxicity was determined using the formula:

$$\% \text{ cytotoxicity} = \left(1 - \frac{A_s}{A_c}\right) \times 100$$

5 wherein  $A_s$  represents the mean absorbance for each treatment and  $A_c$  represents mean absorbance of the control treatment, i.e. cells not exposed to a conjugate.

10 FIG. 1 shows that PEG-TT7-ADR ( $\diamond$ ) and PEG-TT13-ADR ( $\Delta$ ) kill such HSB-2 T cells at concentrations about 100-fold lower than that required for PEG-GFLG-ADR ( $\square$ ) to effect similar levels of cytotoxicity. The cytotoxicities of PEG-TT7-ADR and PEG-TT13-ADR were substantially identical. These results show that the  
15 presence of a ligand specific for binding to the IL-2 receptor and inducing receptor-mediated endocytosis results in much greater cytotoxicity than a PEG- and adriamycin-containing conjugate lacking such ligand. Thus, a conjugate bearing an IL-2-receptor specific  
20 ligand is internalized with much greater efficiency than similar conjugates lacking such a ligand. The unconjugated adriamycin control rapidly diffuses into the cells and kills them. As expected, cytotoxicities from PEG-TT7-ADR and PEG-TT13-ADR require higher  
25 concentrations of adriamycin than unconjugated adriamycin due to the requirement that PEG-TT7-ADR and PEG-TT13-ADR be internalized by endocytosis.

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30 The compositions according to the present invention can be employed for targeted delivery of a

chemical agent to IL-2-receptor-bearing cells, e.g. activated T cells, generally by contacting the cells with the composition under conditions in which binding of the ligand to a receptor stimulates endocytosis of the composition into the cells. The chemical agent then acts on or within the targeted cell into which the composition is internalized, and the desired effect of the active agent can be confined to those cells having the receptor.

For example, a composition according to the invention can be employed as an effective antitumor agent *in vivo* for killing activated T cells. Preferably, the composition is administered to the subject by systemic administration, typically by subcutaneous, intramuscular, or intravenous injection, or intraperitoneal administration, which are methods well known in the art. Injectables for such use can be prepared in conventional forms, either as a liquid solution or suspension or in a solid form suitable for preparation as a solution or suspension in a liquid prior to injection, or as an emulsion. Suitable excipients include, for example, water, saline, dextrose, glycerol, ethanol, and the like; and if desired, minor amounts of auxiliary substances such as wetting or emulsifying agents, buffers, and the like may be added. Effective amounts of such compositions can be determined by those skilled in the art without undue experimentation according to the guidelines provided herein.

The composition can be contacted with the cells *in vitro* or *in vivo*. The T cells constitute a subpopulation of a mixed population of cell types; the ligand according to the invention can provide for endocytosis of the conjugate into T cells and possibly

into a small proportion of other cells having a closely related receptor.

5 The chemical agent can have any of a variety of desired effects in the targeted cells. As mentioned above, in some particularly useful embodiments the chemical agent is effective on a cell only when, or principally when, the agent is internalized into the cell.

10

#### Example 11

##### In Vivo Targeted Delivery to T cells

15 Compositions according to the present invention can be administered to a warm-blooded animal for targeted delivery to IL-2-receptor-bearing cells, e.g. activated T cells. Particularly, the composition provides for receptor-mediated internalization of the composition into the targeted cells.

20 About  $1 \times 10^6$  CCRF-CEM human T-cell leukemia cells in 500  $\mu$ l of PBS were injected intraperitoneally into male CB 17 SCID (HSD)mice, and the cells were allowed to colonize the mice for 24 hours. The human T-cell leukemia cells were found to preferentially colonize the spleen and liver. The mice were divided into 6  
25 groups of 6 animals each: Group A was treated with 100  $\mu$ g of conjugate; Group B were treated with 75  $\mu$ g of conjugate; Group C was treated with 50  $\mu$ g of conjugate; Group D was treated with 25  $\mu$ g of conjugate; Group E was treated with 10  $\mu$ g of  
30 conjugate; and Group F was not treated with any conjugate. After 24 hours, the mice were injected intraperitoneally with 10-100  $\mu$ g of PEG-TT13-ADR (calculated on the mass of ADR in the conjugate) or, in the case of Group F, were not treated. After an

additional 24 and 48 hours, Groups B, C, D, and E were injected again.

Based on previous experiments, it is expected that by 60 days after injection, all of the animals in Group F will die from uncontrolled growth of the T-cell leukemia cells, whereas all of the animals in Groups A-E, i.e. injected with PEG-TT13-ADR, will be alive. These results will show that a composition according to the present invention, wherein the chemical agent is a cytotoxin, preferentially is internalized by IL-2-receptor-bearing cells, i.e. T cells, and such T cells are killed by the cytotoxin.

#### Example 12

In this example, mice are injected with CCRF-CEM human T-cell leukemia cells and with either PEG-TT13-ADR or PEG-GFLG-ADR according to the procedure of Example 11 to determine whether the liver and spleen of such animals contain human cells. The spleen and liver are harvested upon death of the animals or at 120 days post-inoculation, whichever occurs earlier. PCR assay of genomic DNA prepared from these organs is used to determine the presence or absence of human cells therein.

Genomic DNA is prepared from mouse spleen and liver according to methods that are generally well known in the art. See, e.g., J. Sambrook et al., Molecular Cloning: A Laboratory Manual (2d ed., 1989); T. Maniatis et al., Molecular Cloning: A Laboratory Manual (1982); F. Ausubel et al., Current Protocols in Molecular Biology (1987). An illustrative method for preparation of genomic DNA will now be briefly described. The excised spleen and liver are disrupted and the resulting cells are washed in PBS. The cells are then resuspended in a buffer containing 100 mM

NaCl, 10 mM Tris-HCl, pH 8.0, 25 mM EDTA, 0.5% SDS, and 0.1 mg/ml proteinase K, and incubated overnight at 37°C. The resulting lysate is then twice extracted with phenol/chloroform/isoamylalcohol. The DNA in the aqueous phase is then precipitated with ethanol, washed, dried, and resuspended in a buffer containing 10 mM Tris-HCl, pH 8.0, 1 mM EDTA.

PCR is well known in the art for determining the presence of selected sequences in genomic DNA samples.

The following references illustrate PCR methodology:

PCR Technology: Principles and Applications for DNA Amplification (H. Erlich ed., Stockton Press, New York, 1989); PCR Protocols: A Guide to Methods and Applications (Innis et al. eds, Academic Press, San

Diego, Calif., 1990); U.S. Patent Nos. 4,683,195; 4,683,202; 4,800,159; 4,965,188. Briefly, PCR reactions are carried out in glass capillary tubes in

10 µl volumes containing 1.25 mM of each of the four deoxynucleotide triphosphates, 0.72 units of Thermus aquaticus (Taq) DNA polymerase, 35-70 pmol of each primer (20-23 nucleotides in length), 2 µg genomic DNA, and a reaction buffer containing 50 mM Tris-HCl, pH 8.3, 3 mM MgCl<sub>2</sub>, 20 mM KCl, and 0.5 mg/ml of bovine serum albumin. Amplification is routinely carried out

by 60 cycles of PCR. Variations from these parameters, such as the amount of DNA and number of cycles of amplification can be determined empirically by a person of ordinary skill in the art without undue experimentation.

The reaction mixtures are sealed in capillary tubes and then the capillaries are placed in a Model 1605 Air Thermocycler (Idaho Technology, Idaho Falls, Idaho). Parameters of annealing temperature, elongation time, and number of cycles are selected.

Increasing the annealing temperature increases the

specificity of PCR amplification reactions and decreases the amounts of nonspecific products.

Annealing temperature can be estimated from thermal melting temperature according to the formula:

5  $T_m = 4^{\circ}\text{C}(\text{no. of G and C residues in primer}) + 2^{\circ}\text{C}(\text{no. of A and T residues in primer})$ . A person of ordinary skill in the art can optimize the annealing temperature according to known principles. The elongation time depends on the size of product to be amplified. As a rule of thumb, about 4 seconds is sufficient for products of about 100-150 bp, about 8 seconds is sufficient for products of about 200-300 bp, and about 20 seconds is sufficient for products larger than about 500 bp. Increasing elongation times may result in amplification of nonspecific products.

15 After amplification, the reaction mixture is removed from the capillary, mixed with an equal volume of stop solution (95% formamide, 20 mM EDTA, 0.05% bromphenol blue, 0.05% xylene cyanol FF), and either stored frozen or immediately heated at 95°C for 5 minutes and subjected to agarose gel electrophoresis. The fractionated products are then detected by ethidium bromide staining.

25 An illustrative method of determining the relative amounts of human and mouse cells in spleen and liver tissues involves comparison of amplified products from reactions with mouse  $\beta$ -actin and human  $\beta$ -actin specific primers. Illustrative mouse  $\beta$ -actin primers are as follows:

30

GTAACAATGC CATGTTCAAT (SEQ ID NO:22)

CTCCATCGTG GGCCGCTCTA G (SEQ ID NO:23)

Illustrative human  $\beta$ -actin primers are as follows:

CTTAGTTGCG TTACACCCTT TC (SEQ ID NO:24)

35 GGGCCATTCT CCTTAGAGAG AAG (SEQ ID NO:25)



Based on previous experiments, the results of this experiment are expected to show that all of the mice treated with the control PEG-GFLG-ADR composition exhibit the presence of both human and mouse DNA by PCR analysis with specific  $\beta$ -actin primers. Further, mice treated with the PEG-TT13-ADR composition that die within 120 days of administration of the human T cell leukemia cells also exhibit the presence of human DNA. However, mice treated with the PEG-TT13-ADR composition that live for 120 days after administration of the human T cell leukemia cells do not exhibit the presence of human DNA. These results demonstrate that a ligand and cytotoxin-containing composition according to the present invention selectively kills T cells in animals to which it is administered.

#### Example 13

A method of treating T cell lymphoma in a human comprises (a) providing a composition according to the present invention including a ligand, such as the ligand (SEQ ID NO:1) or a biologically functional equivalent thereof, and a cytotoxin, such as adriamycin, both of which are coupled to water soluble polymer, such as PEG, by means of a spacer (Gly-Phe-Leu-Gly; SEQ ID NO:21) and (b) systemically administering an effective amount of the composition to an individual. Such composition can be made, for example, as shown above in Example 1. An effective amount of the composition is systemically administered to the individual such that the composition enters the bloodstream and contacts T cells. The composition binds to an IL-2 receptor on the T cells and stimulates internalization of the composition by endocytosis. The biodegradable spacer is digested by

intracellular proteases, releasing the adriamycin. The adriamycin then kills the cell by intercalating with DNA in the cell. This procedure reduces the number of malignant T cells in the body of the individual, thereby having a positive effect in treatment of the disease.

Claims

We claim:

1. A composition for intracellular delivery of a chemical agent into an IL-2-receptor bearing cell, the composition comprising (a) a water-soluble, biocompatible polymer, (b) the chemical agent covalently, releasably coupled to the polymer, and (c) at least two copies of a ligand comprising an IL-2-receptor-binding peptide covalently coupled to the polymer.

2. The composition of claim 1, wherein the composition has a formula selected from the group consisting of  $P-[T_a-L-S_b-A]_c$  and  $[A-S_b]_d-P-[T_a-L]_c$ , wherein L is the ligand; A is the chemical agent; S and T are spacers, wherein at least S is biodegradable; P is a water soluble polymer having functional groups compatible with forming covalent bonds with the ligand; a and b are integers of 0 or 1; c is an integer of at least 2; and d is an integer of at least 1.

3. The composition of claim 2 wherein P is a polyalkylene oxide.

4. The composition of claim 3 wherein said polyalkylene oxide is a member selected from the group consisting of alpha-substituted polyalkylene oxide derivatives, polyethylene glycol (PEG) homopolymers, polypropylene glycol homopolymers, alkyl-capped polyethylene oxides, bis-polyethylene oxides, copolymers of poly(alkylene oxides), and block copolymers of poly(alkylene oxides) or activated derivatives thereof.

5. The composition of claim 4 wherein said polyalkylene oxide has a molecular weight of about 200 to about 50,000.

5 6. The composition of claim 5 wherein said polyalkylene oxide has a molecular weight of about 2,000 to about 20,000.

7. The composition of claim 6 wherein said  
10 polyalkylene oxide has a molecular weight of about 5,000.

8. The composition of claim 5 wherein said  
15 polyalkylene oxide is an activated polyethylene glycol.

9. The composition of claim 5 wherein said polyalkylene oxide is polyethylene oxide.

20 10. The composition of claim 3 wherein said IL-2-receptor-binding peptide is a member selected from the group consisting of SEQ ID NO:1 and biologically functional equivalents thereof.

25 11. The composition of claim 10 wherein said IL-2 receptor-binding peptide is a member selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:11.

30 12. The composition of claim 10 wherein said chemical agent is selected from the group consisting of cytotoxins, transforming nucleic acids, gene regulators, labels, antigens, and drugs.

13. The composition of claim 12 wherein said spacer comprises a peptide.

14. The composition of claim 13 wherein said  
5 spacer comprises Gly-Phe-Leu-Gly (SEQ ID NO:21).

15. The composition of claim 14 wherein said chemical agent is adriamycin.

10 16. The composition of claim 12 further comprising a carrier selected from the group consisting of other water soluble polymers, liposomes, and particulates.

15 17. The composition of claim 16 wherein said carrier is a water soluble polymer selected from the group consisting of dextran, inulin, poly(L-lysine) with modified epsilon amino groups, poly(L-glutamic acid), and N-substituted methacrylamide-containing  
20 polymers.

18. The composition of claim 1 wherein said IL-2-receptor-bearing cell is an activated T cell.

25 19. A method of delivering a chemical agent in vitro into a IL-2-receptor-bearing cell in a heterogeneous population of cells, comprising the steps of:

30 (a) providing a composition comprising (i) a water-soluble, biocompatible polymer, (ii) the chemical agent covalently, releasably coupled to the polymer, and (iii) at least two copies of a ligand comprising an IL-2-receptor-binding peptide covalently coupled to the polymer; and

(b) contacting the population of cells with an effective amount of the composition under conditions wherein the ligand binds to an IL-2 receptor on the IL-2-receptor-bearing cells and elicits endocytosis of the composition.

20. The method of claim 19, wherein the composition has a formula selected from the group consisting of  $P-[T_a-L-S_b-A]_c$  and  $[A-S_b]_d-P-[T_a-L]_c$ , wherein L is the ligand; A is the chemical agent; S and T are spacers, wherein at least S is biodegradable; P is a water soluble polymer having functional groups compatible with forming covalent bonds with the ligand; a and b are integers of 0 or 1; c is an integer of at least 2; and d is an integer of at least 1.

21. The method of claim 20 wherein P is a polyalkylene oxide.

22. The method of claim 21 wherein said polyalkylene oxide is a member selected from the group consisting of alpha-substituted polyalkylene oxide derivatives, polyethylene glycol (PEG) homopolymers, polypropylene glycol homopolymers, alkyl-capped polyethylene oxides, bis-polyethylene oxides, copolymers of poly(alkylene oxides), and block copolymers of poly(alkylene oxides) or activated derivatives thereof.

23. The method of claim 22 wherein said polyalkylene oxide has a molecular weight of about 200 to about 50,000.

24. The method of claim 23 wherein said polyalkylene oxide has a molecular weight of about 2,000 to about 20,000.

5           25. The method of claim 24 wherein said polyalkylene oxide has a molecular weight of about 5,000.

10           26. The method of claim 23 wherein said polyalkylene oxide is an activated polyethylene glycol.

15           27. The method of claim 23 wherein said polyalkylene oxide is polyethylene oxide.

20           28. The method of claim 21 wherein said IL-2-receptor-binding peptide is a member selected from the group consisting of SEQ ID NO:1 and biologically functional equivalents thereof.

25           29. The method of claim 28 wherein said IL-2 receptor-binding peptide is a member selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:11.

30           30. The method of claim 28 wherein said chemical agent is selected from the group consisting of cytotoxins, transforming nucleic acids, gene regulators, labels, antigens, and drugs.

35           31. The method of claim 30 wherein said spacer comprises a peptide.

            32. The method of claim 31 wherein said spacer comprises Gly-Phe-Leu-Gly (SEQ ID NO:21).

33. The method of claim 32 wherein said chemical agent is adriamycin.

34. The method of claim 30 further comprising a carrier selected from the group consisting of other water soluble polymers, liposomes, and particulates.

35. The method of claim 34 wherein said carrier is a water soluble polymer selected from the group consisting of dextran, inulin, poly(L-lysine) with modified epsilon amino groups, poly(L-glutamic acid), and N-substituted methacrylamide-containing polymers.

36. The method of claim 19 wherein said IL-2-receptor-bearing cell is an activated T cell.

37. A method of delivering a chemical agent intracellularly into an IL-2-receptor-bearing cell in a warm-blooded animal, comprising the steps of:

(a) providing a composition comprising (i) a water-soluble, biocompatible polymer, (ii) the chemical agent covalently, releasably coupled to the polymer, and (iii) at least two copies of a ligand comprising an IL-2-receptor-binding peptide covalently coupled to the polymer; and

(b) systemically administering to said warm-blooded animal an effective amount of said composition under conditions wherein said ligand contacts and binds to an IL-2 receptor on the IL-2-receptor-bearing cell and elicits endocytosis of said composition.

38. The method of claim 37, wherein the composition has a formula selected from the group consisting of  $P-[T_a-L-S_b-A]_c$  and  $[A-S_b]_d-P-[T_a-L]_e$ , wherein L is the ligand; A is the chemical agent; S



and T are spacers, wherein at least S is biodegradable; P is a water soluble polymer having functional groups compatible with forming covalent bonds with the ligand; a and b are integers of 0 or 1; c is an integer of at least 2; and d is an integer of at least 1.

39. The method of claim 38 wherein P is a polyalkylene oxide.

40. The method of claim 39 wherein said polyalkylene oxide is a member selected from the group consisting of alpha-substituted polyalkylene oxide derivatives, polyethylene glycol (PEG) homopolymers, polypropylene glycol homopolymers, alkyl-capped polyethylene oxides, bis-polyethylene oxides, copolymers of poly(alkylene oxides), and block copolymers of poly(alkylene oxides) or activated derivatives thereof.

41. The method of claim 40 wherein said polyalkylene oxide has a molecular weight of about 200 to about 50,000.

42. The method of claim 41 wherein said polyalkylene oxide has a molecular weight of about 2,000 to about 20,000.

43. The method of claim 42 wherein said polyalkylene oxide has a molecular weight of about 5,000.

44. The method of claim 41 wherein said polyalkylene oxide is an activated polyethylene glycol.

45. The method of claim 41 wherein said polyalkylene oxide is polyethylene oxide.

5 46. The method of claim 39 wherein said IL-2-receptor-binding peptide is a member selected from the group consisting of SEQ ID NO:1 and biologically functional equivalents thereof.

10 47. The method of claim 46 wherein said IL-2 receptor-binding peptide is a member selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:11.

15 48. The method of claim 46 wherein said chemical agent is selected from the group consisting of cytotoxins, transforming nucleic acids, gene regulators, labels, antigens, and drugs.

20 49. The method of claim 48 wherein said spacer comprises a peptide.

50. The method of claim 49 wherein said spacer comprises Gly-Phe-Leu-Gly (SEQ ID NO:21).

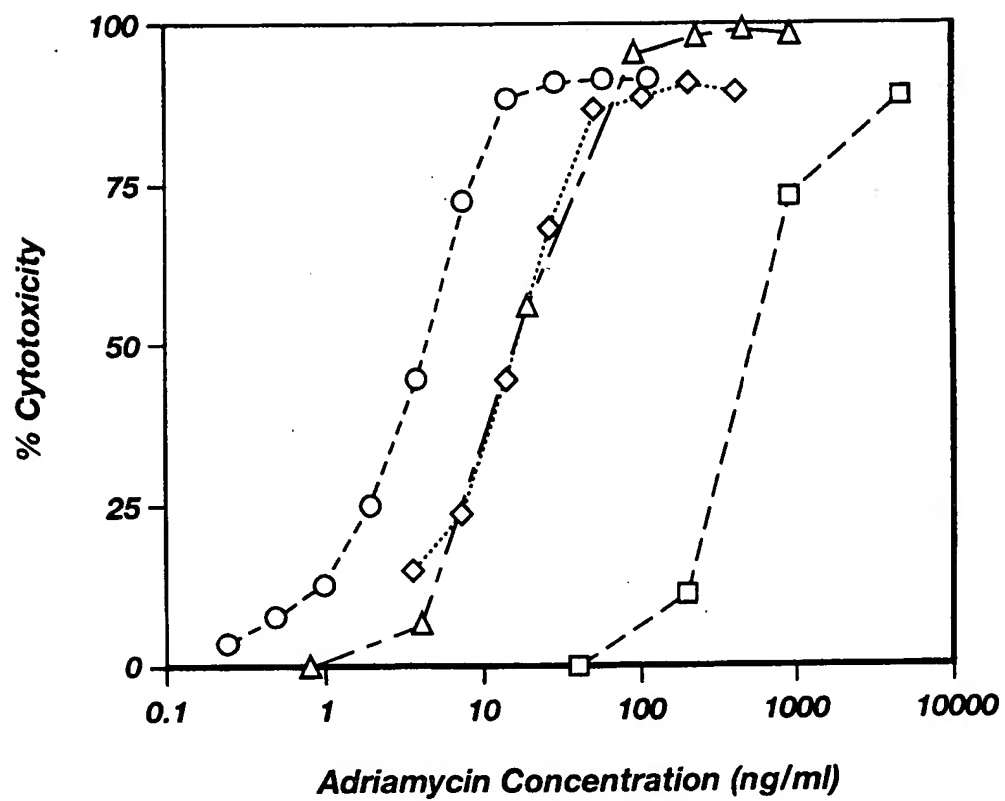
25 51. The method of claim 50 wherein said chemical agent is adriamycin.

52. The method of claim 48 further comprising a carrier selected from the group consisting of other water soluble polymers, liposomes, and particulates.

30 53. The method of claim 52 wherein said carrier is a water soluble polymer selected from the group consisting of dextran, inulin, poly(L-lysine) with modified epsilon amino groups, poly(L-glutamic acid),  
35 and N-substituted methacrylamide-containing polymers.

54. The method of claim 37 wherein said IL-2-receptor-bearing cell is an activated T cell.

1/1

**Fig. 1**

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